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THE PROTECTIVE AND CURATIVE VALUE OF QUININ AND UREA HYDROCHLORID, ETHYLHYDROCUPREIN, AND OTHER CINCHONA DERIVATIVES IN EXPERIMENTAL PNEUMOCOCCUS INFECTIONS *

STUDIES IN PNEUMONIA, III

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In previous communications[1, 2] we have summarized the results of our studies, with various methods, on the bactericidal action of various cinchonics on pneumococci in vitro, showing that these substances as a group, and ethylhydrocuprein in particular, exert a specific and relatively high bactericidal action on type strains of this microorganism. The object of the present paper is to present briefly the results of our studies concerning the toxicity of these compounds for animals and their protective and curative influence with respect to experimental pneumococcus infections produced by the intraperitoneal and intravenous injection of highly virulent cultures.

The empirical use of cinchona and of quinin compounds in the treatment of acute pneumonia is old, and the power of these agents, when given in sufficient dose sufficiently early, to arrest the progress of pneumonic toxemia, even tho the accompanying tissue changes may not be materially affected, is attested by numerous observers whose conclusions have indeed been disputed, but whose general competency as diagnosticians and pathologists cannot be questioned. In a previous paper[1] we have cited some of these observations.

Influenced less by this tradition than by the effects of the use of quinin compounds as observed in experimental trypanosomiasis, and by the fact that certain peculiar biologic relationships (as bile solubility) have been found by Schilling[3] and Neufeld[4, 5] to exist between trypanosomes and spirilla on the one hand and pneumococci on the other, Morgenroth and Levy[6] chose quinin and its derivatives as a basis and lead for chemotherapeutic studies on experimental

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* Received for publication November 27, 1916.

[1] Cohen, Kolmer, and Heist, Jour. Infect. Dis., 1917, 20, p. 40.

[2] Kolmer, Cohen, and Heist, ibid., p. 81.

[3] Centralbl. f. Bakteriol., I, O., 1902, 31, p. 452.

[4] Arb. a. d. k. Gsndhtsamte, 1907, 25, p. 494.

[5] Ztschr. f. Hyg. u. Infektionskr., 1900, 34, p. 454.

[6] Berl. klin. Wchnschr., 1911, 48, pp. 1560, 1979.

pneumococcus infections. Best results were observed with optochin (ethylhydrocuprein) a synthetic derivative of hydroquinin (methylhydrocuprein) which latter exists in the cinchona bark or can be prepared synthetically.* In experimental infections of mice the new drug (optochin) was found to prevent the development of infection in some 90% of animals in which treatment was postponed till after inoculation. Later Guttman,[7] Morgenroth and Kaufman,[8] and Levy[9] reported further experiments indicating the protective and curative action of ethylhydrocuprein (base) and its hydrochlorid, in experimental infections in mice. Wright[10] found that the administration of ethylhydrocuprein to man resulted in an increased bactericidal power of the blood. These observations were confirmed by Moore[11] with animals. Moore[12] also reported that ethylhydrocuprein exerts a well-marked protective action against experimental pneumococcal infection in mice in the case of type strains of all four groups of pneumococci; this protective action was found efficient against many multiples of the minimal lethal dose. Rosenthal[13] gives an excellent review of the literature bearing on the chemotherapeutic treatment of pneumonia, leading up to and including the work of Morgenroth and Levy and others.

Clinically, the local application of a 1-2% solution of ethylhydrocuprein hydrochlorid has been reported by a number of opbthalmologists as efficacious in the treatment of pneumococcus ulcers of the conjunctiva and cornea tho somewhat irritating. In the treatment of lobar pneumonia the consensus of opinion would seem to indicate that the drug is not of proved value and particularly must care be exercised in the dosage to avoid the amblyopia which has been reported by a number of investigators. The general statement is made that there is but "a small interval between the therapeutic and the toxic dose."

Smith and Fantus[14] have reported that the minimal lethal dose of ethylhydrocuprein for mice is 0.5 mg. per gram of body weight; the dose for quinin is given as slightly higher or 0.7 mg. per gram of body weight. They found that optochin will reduce blood pressure more than quinin and cause quicker cardiac death. Hunt[15] found the lethal dose of hydroquinin to be from 0.35 to 0.33 mg. per gram, and this was confirmed by Morgenroth and Halberstädter.[16]

Our own results follow.

THE LETHAL DOSE

The smallest immediately lethal doses of ethylhydrocuprein hydrochlorid and 3 quinin salts were determined for mice and rabbits by intravenous and intramuscular injection.

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* The chemical relationship of the cuprein group to the quinin group is indicated by the following formulas: Cuprein, $C_{19}H_{20}N_2.OH.OH$; Quinin, $C_{19}H_{20}N_2.OH.OCH_3$; Hydrocuprein, $C_{19}H_{22}N_2.OH.OH$; Methylhydrocuprein (Hydroquinin), $C_{19}H_{22}N_2.OH.OCH_3$; Ethyl Hydrocuprein, $C_{19}H_{22}NOH.OC_2H_5$; Quinethylin (according to Grimaux and Arnaud), $C_{19}H_{22}N_2.OH.OC_2H_5$.

[7] Ztschr. f. Immunitätsf., 1912, 15, p. 625.

[8] Centralbl. f. Bakteriol., R., 1912, 54, p. 69.

[9] Berl. klin. Wchnschr., 1912, 49, p. 2486.

[10] On Pharmaco-therapy and Preventive Inoculation Applied to Pneumonia in the African Native. 1915. (This little book gives a complete account of the investigations of the author and his associates with ethylhydrocuprein and vaccines in human pneumococcus infections).

[11] Jour. Exper. Med., 1915, 22, p. 551.

[12] Ibid., p. 269.

[13] Ztschr. f. Chemotherap., R., 1912, 1, p. 1149.

[14] Jour. Pharmacol. and Exper. Therap., 1916, 8, p. 53.

[15] Arch. internat. de Pharmacod. et de Thérap., 1904, 12, p. 503.

[16] Sitzungsberichte der Königl. Preuss. Akademie der Wissenschaften (Physikal.-mathemat. Classe), 1911, 1, p. 34.

Lethal Dose by Intravenous Injection.—The results of a series of experiments are summarized in Table 1.

All the drugs were dissolved in sterile distilled water and filtered before injection. The total amount of fluid injected did not exceed 1 c.c. for mice or 5 c.c. for rabbits, and in all instances the rate of injection was such as to require about 5 seconds per cubic centimeter. More rapid injection of ethylhydrocuprein tended to increase its toxic effects. Mice were injected by means of a vein in the tail and rabbits by a marginal vein of the ear.

In mice the effects were first evidenced by attempts to switch the tail, muscular twitchings in all extremities, and death after a brief convulsion. Rabbits either develop spasmodic movements of the hind legs, and then of the forelegs, followed by weakness and complete paralysis of the limbs, dyspnea, collapse, and death, or at once develop a severe convulsion with exophthalmos, and die in a few seconds.

TABLE 1

LETHAL DOSE OF ETHYLHYDROCUPREIN AND OTHER CINCHONA DERIVATIVES BY INTRAVENOUS INJECTION

Substance	Animal	Lethal Dose		Estimated Dose per 60 Kilos (125 lb.)
		Per Gram	Per Kilo	
Ethylhydrocuprein hydrochlorid........	Mouse.....	.00006	.06	3.6
	Rabbit....	.00006	.06	3.6
Quinin and urea hydrochlorid..........	Mouse.....	.00006	.06	3.6
	Rabbit....	.00005	.05	3.0
Hydroquinin hydrochlorid..............	Mouse.....	.00004	.04	2.4
	Rabbit....	.00005	.05	3.0
Quinin hydrochlorid....................	Mouse.....	.00006	.06	3.6
	Rabbit....	.00005	.05	3.0

The immediately lethal doses given in the table represent the average results observed with mice weighing from 14 to 22 gm. and rabbits weighing from 1200 to 2000 gm. In all instances each dose was given strictly according to body weight. All animals were kept in the laboratory at a temperature varying from 67 to 76 F. Individual animals exhibited varying degrees of resistance; as in all biologic experiments, mathematically accurate and constant results according to dosage per body weight are not possible.

Summary.—The immediate lethal dose of ethylhydrocuprein hydrochlorid was 0.06 gm. and of the other cinchonics, from 0.04 to 0.06 gm., per kilo of body weight. In other words, the toxicity of ethylhydrocuprein hydrochlorid was equal to or slightly greater than that of the other compounds tested. Hydroquinin (the parent of ethylhydrocuprein) appeared slightly more toxic than the other compounds — a result not in accord with the majority of reported observations.

As a general rule, mice withstood slightly larger doses than rabbits. Individual experiments also indicated that young rabbits were more resistant than older and heavier animals, confirming the observations of Moore[11] in this respect.

Lethal Dose by Intramuscular Injection.—The results of a series of experiments are shown in Table 2. The results were more irregular than observed with the intravenous route; the doses given are those which proved lethal in the majority of tests.

TABLE 2

LETHAL DOSE OF ETHYLHYDROCUPREIN AND OTHER CINCHONA DERIVATIVES BY SINGLE INTRAMUSCULAR INJECTION

Substance	Animal	Lethal Dose		Estimated Dose per 60 Kilos (125 lb.)
		Per Gram	Per Kilo	
Ethylhydrocuprein hydrochlorid........	Mouse..... Rabbit....	.0006 .0005	.6 .5	36.0 30.0
Quinin and urea hydrochlorid..........	Mouse..... Rabbit....	.0005 .0005	.5 .5	30.0 30.0
Hydroquinin hydrochlorid..............	Mouse.....	.00016- .00025	.16-.25	12.3
Quinin hydrochlorid....................	Mouse..... Rabbit....	.0005 .0005	.5 .5	30.0 30.0

Mice were injected in the muscles of the thigh; rabbits in the erector spinæ and thigh muscles (usually the latter).

Summary.—The lethal doses of these compounds by intramuscular injection were in general about 10 times larger than the lethal doses by intravenous injection.

Mice withstood slightly larger doses of ethylhydrocuprein hydrochlorid than rabbits.

The toxicity of ethylhydrocuprein hydrochlorid was generally slightly greater than that of the quinin compounds, except in the case of hydroquinin, which proved more toxic for mice than the other compounds.

THE TOLERATED DOSE

The largest tolerated dose of ethylhydrocuprein hydrochlorid and of 3 other cinchona derivatives was determined in a series of experiments on mice and rabbits by intravenous, intramuscular, and subcutaneous injection.

Moore[11] has reported that rabbits weighing approximately 2000 gm.

tolerate a single dose of 0.1 gm. of ethylhydrocuprein hydrochlorid subcutaneously and 0.02 to 0.05 gm. per kilo of body weight, when it is given slowly, intravenously.

All drugs were dissolved in sterile distilled water and the technic was the same as that described. All animals were observed for a period of 10 days following the injections. After this time deaths occurred at irregular intervals and it was frequently impossible to determine the exact cause. For the sake of definiteness, therefore, we have given the results as observed after this relatively short interval.

In the tables the smaller and larger doses as found in different experiments, are given; the estimated dose per 60 kilos is based on an average of these.

The results of experiments are summarized in Tables 3, 4, and 5.

Summary.—Ethylhydrocuprein hydrochlorid was tolerated in about the same dosage as the quinin compounds or was slightly more toxic.

As a general rule, mice withstood slightly larger doses than rabbits.

The tolerated intramuscular dose of these compounds was about one-half greater than the intravenous dose.

The tolerated subcutaneous dose was about 4 times greater than the intravenous dose, and from 2 to 3 times greater than the intramuscular dose.

PROTECTIVE VALUE

Experiments on the protective value of quinin and urea hydrochlorid, ethylhydrocuprein, and other cinchonics were conducted by administering the drugs first, and then inoculating the animals by intraperitoneal or intravenous injection of multiples of the minimal lethal doses of 24-hour cultures.

Strains of pneumococci belonging to Types I, II, and III were employed; most of our work was conducted with Type I. The minimal lethal doses of these strains were determined by frequent intraperitoneal injection into mice and intravenous injection into rabbits and within 2 days of the time selected for the experiments. Their virulence for mice of about 20 gm. in weight and rabbits of about 1600 gm. was generally at or near the following amounts of 24-hour blood-dextrose-broth cultures:

Type I (mice) 48 hours: 0.000 001 c.c.
(rabbits) 72 hours: 0.001 to 0.01 c.c.
Type II (mice) 48 hours: 0.000001 c.c.
(rabbits) 72 hours: 0.002 to 0.02 c.c.
Type III (mice) 48 hours: 0.001 c.c.
(rabbits) 72 hours: 0.1 to 0.2 c.c.

According to body weight, mice were considerably more susceptible to all strains than rabbits.

TABLE 3

THE TOLERATED DOSE OF ETHYLHYDROCUPREIN AND OTHER CINCHONA DERIVATIVES BY INTRAVENOUS INJECTIONS

Substance	Animal	Tolerated Dose		Estimated Dose per 60 Kilos (125 lb.)
		Per Gram	Per Kilo	
Ethylhydrocuprein hydrochlorid........	Mouse.....	.00002-.00004	.02-.04	1.8
	Rabbit....	.00002-.00003	.02-.03	1.5
Quinin and urea hydrochlorid..........	Mouse.....	.00002-.00007	.02-.07	2.7
	Rabbit....	.00002-.00005	.02-.05	2.4
Hydroquinin hydrochlorid..............	Mouse.....	.00002-.00006	.02-.06	2.4
	Rabbit....	.00002-.00004	.02-.04	1.8
Quinin hydrochlorid.....................	Mouse.....	.00002-.00005	.02-.05	2.1
	Rabbit....	.00002-.00003	.02-.03	1.5

TABLE 4

THE TOLERATED DOSE OF ETHYLHYDROCUPREIN AND OTHER CINCHONA DERIVATIVES BY SINGLE INTRAMUSCULAR INJECTION

Substance	Animal	The Tolerated Dose		Estimated Dose per 60 Kilos (125 lb.)
		Per Gram	Per Kilo	
Ethylhydrocuprein hydrochlorid........	Mouse.....	.00003 -.00005	.03 -.05	2.4
	Rabbit....	.00003 -.00007	.03 -.07	3.0
Quinin and urea hydrochlorid..........	Mouse.....	.00003 -.00007	.03 -.07	3.0
	Rabbit....	.00003 -.00008	.03 -.08	3.3
Hydroquinin hydrochlorid..............	Mouse.....	.000013-.00004	.013-.04	1.6
	Rabbit....	.00002 -.00006	.02 -.06	2.4
Quinin hydrochlorid.....................	Mouse.....	.00003 -.00006	.03 -.06	2.7
	Rabbit....	.00003 -.00007	.03 -.07	3.0

TABLE 5

THE TOLERATED DOSE OF ETHYLHYDROCUPREIN AND OTHER CINCHONA DERIVATIVES BY SINGLE SUBCUTANEOUS INJECTION

Substance	Animal	Tolerated Dose		Estimated Dose per 60 Kilos (125 lb.)
		Per Gram	Per Kilo	
Ethylhydrocuprein hydrochlorid........	Mouse.....	.00008-.0001	.08-.1	5.4
	Rabbit....	.00008-.00012	.08-.12	6.0
Quinin and urea hydrochlorid..........	Mouse.....	.00009-.00012	.09-.12	6.3
	Rabbit....	.00009-.00015	.09-.15	7.2
Hydroquinin hydrochlorid..............	Mouse.....	.00009-.0001	.09-.1	5.7
	Rabbit....	.00008-.0001	.08-.1	5.4
Quinin hydrochlorid.....................	Mouse.....	.00009-.00012	.09-.12	6.3
	Rabbit....	.00009-.00018	.09-.18	8.1

Animals were infected with from 10 to 1000 times the M.L.D. of the various strains, but our best and most definite results were observed with 50 M.L.D. of a culture and most of the work was conducted with this amount.

All of the drugs were prepared in sterile distilled water, filtered, and sterilized in the Arnold sterilizer.

All the animals were kept under observation for a period of at least 6 days. Examinations were made of the majority of animals succumbing, and the blood of the heart was examined by smear and culture for pneumococci.

Intravenous Administration of Drugs.—The results of a few typical experiments in which the drugs were given intravenously 2 hours before infection are shown in Tables 6, 7, 8, and 9.

TABLE 6

PROTECTIVE VALUE OF ETHYLHYDROCUPREIN ADMINISTERED INTRAVENOUSLY 2 HOURS BEFORE INFECTION*

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	12	.00004	.04	Living on 6th day
2	14	.00003	.03	
3	14	.00002	.02	
4	19	.00001	.01	Died on 5th day
5	17	.000008	.008	Died on 3rd day
6	17	.000006	.006	Died on 3rd day
7	18	.000004	.004	Died on 2nd day
8	23	.000002	.002	
9	16	Controls		
10	18			
11	18			
12	16			

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 7

PROTECTIVE VALUE OF HYDROQUININ HYDROCHLORID ADMINISTERED INTRAVENOUSLY 2 HOURS BEFORE INFECTION *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	20	.00004	.04	Living on 6th day
2	21	.00003	.03	
3	14	.00002	.02	
4	20	.00001	.01	Died on 4th day
5	19	.000008	.008	Died on 3rd day
6	18	.000006	.006	
7	20	.000004	.004	
8	21	.000002	.002	
9	21	Controls		
10	21			Died on 2nd day
11	22			Died on 3rd day
12	19			Died on 2nd day

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 8

PROTECTIVE ACTION OF QUININ AND UREA HYDROCHLORID ADMINISTERED INTRAVENOUSLY 2 HOURS BEFORE INFECTION *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	19	.00004	.04	Died on 4th day
2	19	.00003	.03	Died on 3rd day
3	20	.00002	.02	Died on 3rd day
4	17	.00001	.01	Died on 2nd day
5	15	.000008	.008	
6	16	.000006	.006	
7	17	.000004	.004	
8	19	.000002	.002	
9	13	Controls		Died on 3rd day
10	16			Died on 2nd day
11	21			
12	15			

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 9

PROTECTIVE AND CURATIVE ACTION OF ETHYLHYDROCUPREIN HYDROCHLORID AND OTHER CINCHONA DERIVATIVES ADMINISTERED TO RABBITS BY INTRAVENOUS INJECTION *

Test	Weight (gm.)	Substance	Dose per Kilo	Time of Injection in Relation to Inoculation	Results
1	1590	Ethylhydrocuprein	.01 gm.	4 hr. before	Living on 6th day
2	1140			2 hr. before	Living on 6th day
3	1275			Simultaneously.	Died on 6th day
4	1705			1 hr. after	Died on 4th day
5	1240			2 hr. after	Died on 5th day
6	1490	Quinin and urea hydrochlorid		4 hr. before	Died on 3rd day
7	1350			2 hr. before	Died on 3rd day
8	1275			Simultaneously.	Died on 5th day
9	1320			1 hr. after	Died on 3rd day
10	1275			2 hr. after	Died on 3rd day
11	1350	Hydroquinin hydrochlorid		4 hr. before	Died on 5th day
12	1275			2 hr. before	Died on 4th day
13	1100			Simultaneously.	Died on 3rd day
14	1400			1 hr. after	Died on 3rd day
15	1250			2 hr. after	Died on 3rd day
16	1400	Controls			Died on 2nd day
17	1350				Died on 3rd day
18	1275				Died on 3rd day

* Rabbits infected by intravenous injection with 50 M.L.D. of pneumococcus, Type I.

The results of these and similar experiments in which the animals were infected with 50 M.L.D. of culture of Type I may be summarized as follows:

Of the three cinchonics used, ethylhydrocuprein hydrochlorid showed the most marked protective action against virulent pneumococci. Among mice, doses of ethylhydrocuprein hydrochlorid corresponding to 0.02 gm. per kilo of body weight given 2 hours before infection protected all animals over a period of 6 days and usually much longer. The administration to rabbits of 0.01 gm. per kilo of body weight yielded similar protection.

Among mice, doses of ethylhydrocuprein hydrochlorid corresponding to 0.01-0.006 gm. per kilo of body weight prolonged life for from 1 to 3 or 4 days and did not protect indefinitely.

As a general rule, when the drug in dose of 0.02 gm. for mice and 0.01 gm. for rabbits per kilo and the pneumococci were injected within a few seconds of each other (marked "simultaneously" in the tables) the life of about 20% of the animals was prolonged indefinitely, while in the balance life was prolonged but a few days beyond that of the controls.

Both hydroquinin hydrochlorid and quinin and urea hydrochlorid showed protective value. Hydroquinin proved somewhat superior to quinin and urea hydrochlorid, and in some experiments 0.02 gm. of this salt per kilo of body weight protected mice over a period of 6 days or longer (Table 7). The percentage of protections of this degree was less than observed with ethylhydrocuprein. A dose of 0.01 gm. per kilo of body weight afforded some protection in a small percentage of mice, but smaller doses were without appreciable effect. Among rabbits the administration of hydroquinin in dose of 0.01 gm. per kilo of body weight from 2 to 4 hours previous to inoculation with pneumococci, tended to prolong life for from 1 to 4 days, but when both were given simultaneously practically no protection was afforded.

Quinin and urea hydrochlorid in large doses (0.02 to 0.04 gm. per kilo of body weight) prolonged the life of mice from 1 to 3 or 4 days in the majority of instances; smaller doses were without effect. This double salt in dose of 0.01 gm. per kilo of body weight in rabbits was practically without protective value when given from 4 hours to a few seconds before inoculation with 50 M.L.D. of pneumococcus culture.

Results with Single Intramuscular Injection.—The results of a number of experiments conducted with ethylhydrocuprein hydrochlorid, hydroquinin hydrochlorid, and quinin and urea hydrochlorid administered by single intramuscular injection are shown in Tables 10, 11, 12, and 13.

Summary.—Ethylhydrocuprein hydrochlorid in doses of from 0.02 to 0.06 gm. per kilo of body weight of mice afforded protection in the majority of experiments over a period of 6 days. In general, the

TABLE 10

PROTECTIVE VALUE OF ETHYLHYDROCUPREIN HYDROCHLORID ADMINISTERED IN SINGLE INTRAMUSCULAR DOSE 2 HOURS BEFORE INFECTION *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	19	.00006	.06	Living on 6th day
2	19	.00005	.05	Living on 6th day
3	20	.00004	.04	Died on 3rd day
4	19	.00002	.02	Died on 2nd day
5	17	.00001	.01	
6	20	.000008	.008	
7	21	.000006	.006	
8	19	.000004	.004	
9	20	Controls		
10	21			
11	18			
12	19			

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 11

PROTECTIVE VALUE OF HYDROQUININ HYDROCHLORID ADMINISTERED IN SINGLE INTRAMUSCULAR DOSE 2 HOURS BEFORE INFECTION *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	20	.00006	.06	Died on 3rd day
2	21	.00005	.05	Died on 2nd day
3	18	.00004	.04	
4	23	.00002	.02	
5	15	.00001	.01	
6	18	.000008	.008	
7	21	.000006	.006	
8	16	.000004	.004	
9	20	Controls		
10	21			
11	18			
12	19			

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 12

PROTECTIVE VALUE OF QUININ AND UREA HYDROCHLORID ADMINISTERED IN SINGLE INTRAMUSCULAR DOSE 2 HOURS BEFORE INFECTION *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	15	.00006	.06	Died on 2nd day
2	17	.00004	.04	
3	15	.00003	.03	
4	21	.00002	.02	
5	17	.00001	.01	
6	20	.000008	.008	
7	15	.000006	.006	
8	18	.000004	.004	
9	16	Control		
10	19	Control		
11	21	Control		
12	18	Control		

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 13

PROTECTIVE AND CURATIVE ACTION OF ETHYLHYDROCUPREIN HYDROCHLORID AND OTHER CINCHONA DERIVATIVES ADMINISTERED BY INTRAMUSCULAR INJECTION IN MICE *

Test	Weight (gm.)	Substance	Dose per Kilo	Time of Injection in Relation to Inoculation	Results
1	14	Ethylhydrocuprein	.02 gm.	2 hr. before	Living on 6th day
2	18			Simultaneously.	Died on 5th day
3	12			2 hr. after	Died on 4th day
4	17			6 hr. after	Died on 1st day
5	15	Quinin and urea hydrochlorid		2 hr. before	Died on 5th day
6	16			Simultaneously.	Died on 2nd day
7	12			2 hr. after	Died on 1st day
8	22			6 hr. after	Died on 1st day
9	11	Hydroquinin hydrochlorid		2 hr. before	Died on 6th day
10	14			Simultaneously.	Died on 2nd day
11	19			2 hr. after	Died on 1st day
12	15			6 hr. after	Died on 1st day
13	24	Quinin bisulfate		2 hr. before	Died on 3rd day
14	18			Simultaneously.	Died on 2nd day
15	18			2 hr. after	Died on 1st day
16	19			6 hr. after	Died on 1st day
17	20	Controls			Died on 1st day
18	14				
19	16				
20	19				

* Mice infected by intraperitoneal injection with 10 M.L.D. of pneumococcus, Type I.

degree of protection was less than that observed when the drug was given intravenously. When the drug and pneumococci were given simultaneously doses of 0.04 gm., or higher, per kilo of body weight usually protected; doses of 0.02 gm., or slightly less, prolonged life for a few days among the majority of mice.

Hydroquinin hydrochlorid and quinin and urea hydrochlorid in doses of from 0.02 to 0.06 gm. per kilo of body weight tended to prolong life, and in a few instances the larger doses afforded protection over a period of at least 6 days; but both were inferior to ethylhydrocuprein hydrochlorid and the effects were much less marked than those observed when the drugs were given intravenously.

When these drugs in doses of 0.02 gm., or less, per kilo of body weight were given simultaneously with the pneumococci (50 M.L.D.), practically no protection followed.

A few experiments with quinin bisulfate showed a feeble protective value when given from 2 to 4 hours before the pneumococci.

CURATIVE VALUE

The curative action of ethylhydrocuprein hydrochlorid and other quinin derivatives was studied by injecting the drugs intravenously and intramuscularly at varying intervals after the animals had been inoculated with pneumococci or, in other words, after the infection had had a start.

Otherwise these experiments were conducted in exactly the same manner as the experiments for protective value.

Results with Intravenous Injection.—The results of a number of experiments are shown in Tables 9, 14, 15, 16, and 17.

Summary. — Our percentage of 'cures' with ethylhydrocuprein hydrochlorid when the drug was given within 2 hours after inoculation with 50 M.L.D. of culture was less than 10%. Doses ranging from 0.01 to 0.03 gm. per kilo of body weight usually prolonged life beyond that of the controls from 1 to 4 days, but ultimately the majority of the mice and rabbits succumbed.

Hydroquinin hydrochlorid in amounts as high as 0.04 gm. per kilo of body weight given from 1 to 4 hours after infection with 50 M.L.D. of culture, did not appreciably prolong life except in a few instances when the animals lived from 1 to 3 days longer than the controls.

The double salt, quinin and urea hydrochlorid, was similarly without curative value in doses as high as 0.04 gm. per kilo when given at

TABLE 14

Curative Action of Ethylhydrocuprein Administered Intravenously 2 Hours After Infection *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	16	.00004	.04	Living on 6th day
2	20	.00003	.03	Died on 4th day
3	14	.00002	.02	Died on 2nd day
4	15	.00001	.01	
5	13	.000008	.008	
6	14	.000006	.006	
7	12	.000004	.004	Died on 1st day
8	15	.000002	.002	Died on 2nd day
9	15	Controls		
10	16			
11	18			
12	16			

* Mice infected by intraperitoneal injection with 50 M.L.D. of pneumococcus, Type I.

TABLE 15

Curative Value of Hydroquinin Hydrochlorid Administered Intravenously 2 Hours After Infection *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	17	.00004	.04	Died on 2nd day
2	23	.00003	.03	
3	17	.00002	.02	
4	19	.00001	.01	
5	15	.000008	.008	Died on 5th day
6	22	.000006	.006	Died on 2nd day
7	24	.000004	.004	
8	20	.000002	.002	
9	21	Controls		Died on 3rd day
10	21			Died on 2nd day
11	22			Died on 3rd day
12	19			Died on 2nd day

* Mice infected by intraperitoneal injection with 50 M. L. D. of pneumococcus, Type I.

TABLE 16

Curative Action of Quinin and Urea Hydrochlorid Administered Intravenously 2 Hours After Infection *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	12	.00004	.04	Died on 2nd day
2	21	.00003	.03	
3	20	.00002	.02	
4	21	.00001	.01	
5	19	.000008	.008	Died on 3rd day
6	12	.000006	.006	Died on 2nd day
7	10	.000004	.004	
8	14	.000002	.002	
9	13	Controls		Died on 3rd day
10	16			Died on 2nd day
11	21			
12	15			

* Mice infected by intraperitoneal injection with 50 M. L. D. of pneumococcus, Type II.

TABLE 17

Curative Action of Quinin and Urea Hydrochlorid Administered Intravenously 2 Hours After Infection *

Test	Weight (gm.)	Dose		Results
		Per Gram	Per Kilo	
1	15	.00033	0.33	Died on 6th day
2	19	.00016	0.16	Died on 6th day
3	15	.00008	0.08	Died on 5th day
4	16	.00004	0.04	Died on 5th day
5	18	.00002	0.02	Died on 4th day
6	15	.00001	0.01	Died on 4th day
7	17	.000008	0.008	Died on 3rd day
8	16	.000006	0.006	Died on 2nd day
9	21	Controls		
10	16			
11	17			
12	16			

* Mice infected by intraperitoneal injection with 10 M. L. D. of pneumococcus, Type I.

intervals of an hour or more after inoculation with 50 M.L.D. of culture. When the dose of culture was reduced to 10 M.L.D., a distinct effect was evident in the prolongation of life 4, 5, and 6 days (Table 17) among animals receiving 0.01 gm. and more per kilo of body weight.

Results with Single Intramuscular Injection.—Ethylhydrocuprein in doses ranging from 0.02 to 0.06 gm. per kilo of body weight prolonged the life of a small percentage of mice and rabbits when given within 2 hours after infection with 50 M.L.D. of culture; smaller doses in this time and similar doses given at longer periods were practically without effect (Table 13).

Hydroquinin hydrochlorid, quinin and urea hydrochlorid, and quinin bisulfate in doses ranging as high as 0.04 gm. per kilo of body weight were practically without any effect when given within 1 hour or longer after inoculation with 50 M.L.D. of culture.

Results with Multiple Intramuscular Injection.—Three of the cinchona derivatives were administered by repeated intramuscular injections with results similar to those shown in the 3 experiments detailed in Tables 18, 19, and 20.

Summary.—Ethylhydrocuprein hydrochlorid prolonged the lives of a few more mice to the 7th day or longer than did the other cinchonics. It is probable that the death of some of the mice was due to the cumulative effects of the repeated large doses.

Quinin and urea hydrochlorid ranked second in efficacy to ethylhy-

TABLE 18

RESULTS WITH REPEATED INTRAMUSCULAR INJECTIONS OF ETHYLHYDROCUPREIN HYDROCHLORID AND PNEUMOCOCCUS, TYPE I *

Test	Weight (gm.)	Dose in Each Injection		Number of Injections	Results on 7th Day †
		Per Gram	Per Kilo		
1	18	.00003	.03	4	Died on 5th day
2	22	.000025	.025	7	Living
3	23	.000016	.016	4	Died on 7th day
4	23	.000013	.013	7	Living
5	23	.00001	.01	7	Living
6	19	.000001	.001	6	Died on 7th day
7	21	.000003	.003	7	Living
8	20	.000008	.008	4	Died on 7th day

* Mice infected by intraperitoneal injection with 50 M.L.D. of culture about an hour after the first intramuscular injection of drug.

† Four controls receiving culture alone died within 48 hours.

TABLE 19

RESULTS WITH REPEATED INTRAMUSCULAR INJECTIONS OF QUININ AND UREA HYDROCHLORID AND PNEUMOCOCCUS, TYPE I *

Test	Weight (gm.)	Dose in Each Injection		Number of Injections	Results on 7th Day †
		Per Gram	Per Kilo		
1	18	.00003	.03	3	Died on 4th day
2	17	.000025	.025	5	Died on 5th day
3	18	.000016	.016	7	Living
4	24	.000013	.013	7	Living
5	19	.00001	.01	3	Died on 4th day
6	19	.000005	.006	7	Living
7	21	.000003	.003	7	Living
8	21	.000008	.008	7	Living

* Mice injected by intraperitoneal injection with 50 M. L. D. of culture about an hour after the first intramuscular injection of drug.

† Four controls receiving culture alone died within 48 hours.

TABLE 20

RESULTS WITH REPEATED INTRAMUSCULAR INJECTIONS OF HYDROQUININ HYDROCHLORID AND PNEUMOCOCCUS, TYPE I *

Test	Weight (gm.)	Dose in Each Injection		Number of Injections	Results on 7th Day †
		Per Gram	Per Kilo		
1	22	.00003	.03	7	Died on 4th day
2	24	.000015	.015	3	Died on 3rd day
3	21	.000016	.016	3	Died on 4th day
4	24	.000013	.013	4	Died on 6th day
5	19	.00001	.01	3	Died on 4th day
6	22	.000006	.006	4	Died on 5th day
7	19	.000003	.003	7	Living
8	19	.000008	.008	7	Living

* Mice infected by intraperitoneal injection with 50 M.L.D. of culture about an hour after the first intramuscular injection of drug.

† Four controls receiving culture alone died within 48 hours.

drocuprein, and indeed in the experiment shown (Table 19) carried a larger number (5) of the 8 mice beyond the 7th day.

Hydroquinin hydrochlorid demonstrated a much lower efficacy than the other two compounds. As this derivative is, according to our results, somewhat more toxic for mice and rabbits than the other com-

TABLE 21

RELATION OF TYPE OF PNEUMOCOCCUS TO THE PROTECTION AND CURATIVE ACTION OF QUININ COMPOUNDS *

Test	Weight (gm.)	Substance	Time of Injection in Relation to Inoculation	Type I †				Type II				Type III			
				1	2	3	4	1	2	3	4	1	2	3	4
1	18	Ethylhydrocuprein hydrochlorid	2 hr. before.....				L				L				L
2	16		1 hr. before.....				L				L				D
3	14		Simultaneously.				D				L				D
4	16		1 hr. after......				D				D		D		
5	22		2 hr. after......			D			D				D		
6	18	Hydroquinin hydrochlorid	2 hr. before.....				D				L			D	
7	17		1 hr. before.....			D				D				D	
8	19		Simultaneously.		D				D				D		
9	14		1 hr. after......		D				D				D		
10	18		2 hr. after......		D				D				D		
11	16	Quinin and urea hydrochlorid	2 hr. before.....			D					D			D	
12	16		1 hr. before.....				D			D			D		
13	16		Simultaneously.		D				D				D		
14	20		1 hr. after......	D				D					D		
15	19		2 hr. after......	D				D					D		

D = Died. L = Living.

* Controls on Types I and II died on first day; controls on Type III died within 48 hours. All compounds were given intravenously in dose of 0.02 gm. per kilo of body weight.

† Mice were infected by intraperitoneal injection with 50 M.L.D. of the three types of pneumococci.

pounds, it is highly probable that many of the mice succumbed to the toxic effects of the drug.

RELATION OF TYPE AND VIRULENCE OF PNEUMOCOCCI TO THE PROTECTIVE AND CURATIVE ACTION OF QUININ DERIVATIVES

Cumulative experimentation with virulent cultures belonging to the serologic Types I, II, and III, show that all three are susceptible to the influence of ethylhydrocuprein hydrochlorid and other cinchona derivatives. In several of our experiments, however, Type III was found more resistant (Table 21).

In conducting these comparative tests an attempt was made to infect the animals with as nearly 50 M.L.D. of each type of pneumococcus as possible. The results of one of these comparative tests are shown in Table 18, but similar tests with other cultures belonging to the three types have not yielded exactly similar results, altho, as previously stated, Type III was generally somewhat more difficult to influence.

We have not been able thus far to carry out similar and more extensive experiments by varying the dosage of pneumococci used in infecting the animals. A few comparative experiments conducted with 10, 100, 500, and 1000 minimal lethal doses of Type I show that the protective and curative values of ethylhydrocuprein hydrochlorid and quinin and urea hydrochlorid are considerably influenced by the severity of the infection. As previously stated, the majority of our experiments were conducted with 50 minimal lethal doses of each type, as this dose regularly killed the controls and did not appear excessive for the purpose of obtaining approximately definite results. With greater infection the protective and curative effects were much less noticeable; when 1000 minimal lethal doses were used ethylhydrocuprein was still effective to some degree, but the quinin compounds used were practically without effect.

CONCLUSIONS

The toxicity of ethylhydrocuprein hydrochlorid for mice and rabbits is slightly greater than that of other cinchona derivatives.

As a general rule, mice are found more resistant to the toxic effects of quinin derivatives than rabbits, and young rabbits more than older and heavier rabbits.

The immediately lethal dose of ethylhydrocuprein hydrochlorid in solution by intravenous injection is about 0.06 gm. and of the quinin compounds tested 0.04 to 0.06 gm. per kilo of body weight.

By intramuscular injection the lethal dose of ethylhydrocuprein hydrochlorid is about 0.6 gm. and of the quinin compounds tested about 0.5 gm. per kilo of body weight.

The tolerated dose (period of 10 days) of ethylhydrocuprein hydrochlorid by intravenous injection is about 0.04 gm. and of the quinin compounds tested about 0.05 gm. per kilo of body weight.

By intramuscular injection the tolerated dose of ethylhydrocuprein hydrochlorid is about 0.06 gm. and of the quinin compounds tested about 0.07 gm. per kilo of body weight.

By subcutaneous injection the tolerated dose of ethylhydrocuprein hydrochlorid is about 0.1 gm. and of the quinin compounds tested about 0.12 gm. per kilo of body weight.

In mice the intravenous injection of ethylhydrocuprein hydrochlorid in amount ranging from 0.02 gm. and higher per kilo of body weight affords complete protection against 50 minimal lethal doses of pneumococci given 2 hours later by intraperitoneal injection. Doses ranging from 0.01 to 0.006 gm. afford partial protection.

Among rabbits a dose of at least 0.01 gm. of ethylhydrocuprein per kilo of body weight is required to afford protection against 50 minimal lethal doses of pneumococci given 2 hours later by intravenous injection.

Hydroquinin hydrochlorid and quinin and urea hydrochlorid also possess protective power against virulent pneumococci in mice and rabbits, but to much less degree than ethylhydrocuprein hydrochlorid. Their protective power, however, increases markedly with the dose.

Ethylhydrocuprein hydrochlorid and other cinchona derivatives when intramuscularly administered afford less protection and must be given in larger doses than when administered intravenously.

When ethylhydrocuprein hydrochlorid and the quinin compounds are given at the same time as the pneumococci, protection is much less in evidence.

When ethylhydrocuprein hydrochlorid is given intravenously in amounts ranging from 0.01 to 0.3 gm. per kilo of body weight 2 hours after inoculation with 50 M.L.D. of virulent pneumococci, complete protection, or 'cure,' follows in less than 10% of animals.

Hydroquinin hydrochlorid and quinin and urea hydrochlorid in doses as high as 0.04 gm. per kilo of body weight generally fail to protect, or 'cure,' animals inoculated from 1 to 2 hours earlier with 50 M.L.D. of a culture of virulent pneumococci.

The curative effect of these compounds is less when they are administered by intramuscular injection than when they are given intravenously.

Repeated injections, especially in the case of quinin and urea hydrochlorid, are much more effective than single injections, and in some cases prolong life from 4 to 7 days or longer.

Different serologic types of virulent pneumococci possess in general similar resistance to the effects of ethylhydrocuprein and the quinin compounds. Pneumococci belonging to Type III are, however, not infrequently more resistant than Types I and II.

The results of protective and curative experiments are greatly modified according to the doses of virulent pneumococci used in inoculating the experimental animals, and further observations with smaller but regularly fatal doses of pneumococci may show a greater protective and curative value on the part of an optimal dose of the compounds.

Altho our study shows a real protective and curative value in ethylhydrocuprein in experimental pneumococcus infections, our results demonstrate a somewhat less marked antipneumococcus potency on the part of this drug than has been previously reported.* Other cinchona derivatives are shown to possess also a certain degree of protective power in experimental infections. Further experimentation may improve the bactericidal chemotherapeutic value of ethylhydrocuprein by additions, substitutions, or other molecular modifications. In our opinion it stands today in a position somewhat similar to that of atoxyl in the treatment of experimental trypanosomiasis.

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* It is only fair to point out, however, that both Morgenroth and Moore obtained their best results from the use of optochin base in oil, while the present reports deal chiefly with ethylhydrocuprein hydrochlorid in water. The war has cut off our supply of the drug and we have to await a return of international sanity before we can revise our results in this particular.

Pneumococcus infection in mice and rabbits, however, it needs scarcely be said, differs greatly from the pneumonias of man; and other factors, especially the toxemic complex and the mechanism of crisis (the development of critical immunity and the danger of critical death), have to be considered in the therapeutics of the latter malady. Nevertheless, stress may be laid on the prolongation of life (Table 19) in infected mice receiving repeated injections of nontoxic doses of quinin and urea hydrochlorid—and to a slightly greater extent (Table 18) in those treated with ethylhydrocuprein. For such prolongation in man gives time[17] for the development of the natural immunity which is manifested by critical recovery in untreated cases and by gradual defervescence (lysis) in cases treated with quinin.

It must also be remembered in this connection in so far as the facts here reported may be considered to bear on the value of quinin compounds in the treatment of pneumonias in man — that the use of these agents as advocated by one of us forms but one element in a therapeutic plan,[18] and that, at least until some more potent pneumococcide in vitro than even optochin is found, it is the plan as a whole, and not any single feature only, that is offered for the consideration of practicing physicians.

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[17] Cohen, New York Med. Jour., 1916, 103, p. 1065.

[18] Ibid., p. 1057.